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PRELIMINARY SCREENING OF PATHOGENIC BACTERIA IN CLARIAS BATRACHUS FROM WAINGANGA RIVER OF CHANDRAPUR DISTRICT (M.S.)

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ABSTRACT:
The study was designed to investigate the microbial estimation in the fish *Clarias batrachus* collected from Wainganga river of Chandrapur District. A study was conducted aiming at the isolation of human pathogenic bacteria in gills, intestines, mouth and the skin of apparently healthy fish, *Clarias batrachus*. Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on the season, patients contacted with fish and related environment. Hence an attempt has been made to study the screening of pathogenic bacteria from *Clarias batrachus*. All the bacterial species which were isolated from the fish were also present in the initial water samples collected. The isolation of enteric bacteria in fish serves as indicator organisms of faecal contamination and or water pollution. Their presence also represents a potential hazard to humans. The study revealed that four genera of the pathogenic bacteria were identified belonging to the family *Aeromonas hydrophilia, Enterobacteriaceae family, Escherichia coli* and *Vibrio cholera.*

Keywords :- Clarias batrachus, Enterobacteriaceae, Pathogen, Screening.

INTRODUCTION :

Clarias batrachus is a catfish (Froese, Rainer, and Daniel Pauly, eds.2006). It is highly nourishing and valued as food. It is mostly used in laboratories for experimental purposes but also used as a food. The flesh has high nutritive value and its flesh is said to have wound healing effect and recuperative attributes. It is highly suitable for intensive culture due to its air-breathing habit. In central India it is commonly found in reservoirs of eastern Vidarbha region (M.S.). It is a faster growing fish than most of the other species of the genus. It is marketed live and fetches high prices in the market. Healthy fishes are prized for their table quality. However, this quality is influenced by several operational environmental factors. Suggested that the type of micro-organisms that are found associated with particular fish depends on its habitat classified the bacterial pathogens associated with fish as indigenous and non-indigenous. The non-indigenous contaminate the fish or the habitat one way or the other and examples include *Escherichia coli, Clostridium botulinum, Shigella dynteriae, Staphylococcus aureus, V. cholera* and *Salmonella.*

The studies in the last decade (Karet al., 1993; 1994; 1995; 1996; 1997; 1998; 1999, 2000) showed that species like *Channa striatus, C.punctatus, Clarias batrachus* and *Anabas testudineus* have been severely affected by bacterial pathogens and the outbreak has been occurring during the period from November to March. Low temperatures appear to influence the severity of infectious lesions. The causative agents of the severe acute infectious abdominal dropsy outbreak in Indian major carps. *Cirrhinus mrigala* was reported Shome et al

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(1996). However, the first observation on diseases in Indian major carps was found in descending order of susceptibility on *Catla catla, Cirrhinus mrigala* and *Labeo rohita* (Gopalakrishnan 1981). Other well recorded cases have been the severe epidemic due to the diseased condition of European carps (Snieszko 1954; Van Dujin 1956). Sabur (2006) isolated and identified five species of Aeromonas bacteria in polyculture environment of five carp species *namely Labeo rohita, Cyprinus carpio, Cirrhina scirrhosus, Catla catla* and *Hypophthalmic hthysmolitrix.* Lately the bacteria *A. hydrophila* was isolated from Thai pangus Pangasianodon hypophthalmus (Siddik, 2009) and from climbing perch Anabas testudineus (Sayed, 2010). In the present work, experimental infection was done to know the pathogenicity of bacterial pathoens in *Clarias batrachus.* The virulence of the pathogen was estimated by experimental studies of the LD50 (median lethal dose) of bacterial pathogens in the glass aquaria.

Establishment of *Clarias batrachus* in several continents and its popularity as a freshwater culturable fish species among consumers made the species suitable for meticulous reviews with respect to various parameters. Besides in order to protect the genetic resources of this species from unwanted hybridization, which is very much vulnerable, the fish geneticists and the government bodies should work together. Habitat protection and sustainable consumption of this excellent fish species is the call of the day.

C.batrachus has exceptionally well tolerance level in varied environment that suggests an advantageous evolutionary trait. Toxicity and stress studies can through light on the adaption strategy of a species according to the vibrant changes of the environment and the changed character of the wild habitat, much of which is altered by now due to increasing human intervention and exploitation.

Bacterial flora of some freshwater fishes in tropical water showed that *Aeromonas sp.* was the most predominant microorganism isolated from the skin, gills and intestine of the fish (Karatas Dugenci and Candan., 2003).

Materials and Methods:

Study Area:

This study was conducted on fish species collected from Wainganga River flowing through Gadchiroli and Chandrapur district, Vidharbha (M.S.) In Gadchiroli district the river flows nearby Armori tehsil and in Chandrapur district it is near Bramhapuri tehsil.

Sampling:

The bacterial counts on the external surfaces, intestines and tissue were estimated as follows:

Skin Surfaces:

Sample from different locations of the skin of 40 raw fish was taken by rubbing the sterilized cotton swab over the skin and then inoculated into 9ml of Nutrient broth, MacConkey broth which are dispensed in separate tubes. 10 fold serial dilution of the bacterial suspension inoculated in peptone water was prepared in duplicate and viable aerobic bacterial counts were enumerated using 0.1ml and 1ml inoculums in standard plate count agar as described by (Slaby, B.M., Martin, R.E., Ramsdell, G.E.1981), and then incubated at 37°C for 48 hrs.

Intestines, Gills & Tissues:

1g of the fish sample was dissected out, blended and mixed properly in a mortar. It was aseptically transferred to a sample bottle containing 9mls of 0.1% sterile peptone water The bottle was closed and shaken thoroughly for 10 minutes and allowed to stand for 20 minutes, after which a 10 fold serial dilution was carried out in duplicates and viable aerobic bacterial counts were enumerated in standard plate count agar after incubation at 37°C for 48 hrs

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as described by (Slaby, B.M., Martin, R.E., Ramsdell, G.E.1981).Coliform organisms and gram negative enteric bacteria counts were determined using pour plate method with MacConkey agar, EMB Agar respectively.

Results and Discussion:

In the present study, the isolation and screening of pathogenic bacteria from the infected *Clarius batrachus* from Wianganga rever revealed following observation. In the morphological observation the infected *C. batrachus* showed necrosis and ulceration (Table-1). Based the microbial analysis and the biochemical tests compared with Bergey's Manual revealed the presence of four different bacterial isolates namely *Staphylococcus aureus, Aeromonas hydrophila, Escherichia coli,* and *Vibrio cholera* were isolated. The bacterial composition may change with age, individuals, nutritional status, environmental conditions, and the complexity of the fish. (Table-2 and Table -3) Like all animals, fish suffers from environmental, nutritional and infectious diseases. Organisms such as bacteria and fungi are capable of causing disease under stressful conditions. Intensive and super intensive fish culture practices involve high rates of stocking and supplementary feeding which has substantially enhanced the incidence of disease in fishes in our country.

The identification test results of bacterial isolates from infected fishes are in agreement with previous studies. From the above observation Gram positive and Gram negative bacteria like *Vibrio sp., E. coli, Aeromonas sp.*and *Staphylococcus aureus* was found. Lowered salinity due to rainfall events (Callinan et al., 1995; Virgona, 1992) and excess water discharges appear to play a role subsequent appearance of lesions in fish (Kane et al., 2000). Bodhe and Wadhai (2014) reported microbial infected *Clarias batrachus* from Wardha and Wainganga River during 2011-12 and 2012-13 in winter season. During 2011-2012 out of 69 samples collected from Wardha River, 43 were microbial affected, in 2012-2013, 53 samples out of 83 samples were found to be microbial affected counting to 63.86% infection respectively. However, from Wainganga River 54.22% and 59.09% infected sample were collected during 2011-2012 and 2012-13 respectivelly. Intensive aquaculture of *C.batrachus* in the rural water bodies with very little infrastructure development may bring-about socioeconomic development in many parts of Maharashtra, India. Coordination between government bodies with respect to skill up gradation of the workers, market regulation etc together with the scientific community ensuring timely delivery of better quality seed stock will generate success stories in intensive *Clarias batrachus* culture. Since the species is a part of the natural fauna in this region therefore culture practices will be much easier to follow and therefore much more viable in economic point of view. Regional rural banks and agro finance bodies may be approached for capital requirement and the local governance may forward financial help to the rural entrepreneurs(Surajith, 2011). The present screening was found to be Gram positive and Gram negative bacteria include *Vibrio sp., E. coli, Aeromonas sp.* and *Staphylococcus aureus* are fish infective as well as human pathogenic.

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Table: 1. Colony Morphology and color on different media.

Medium	Colour	Morphology
TCBS Agar	Yellowish Green colour colonies	Rod
EMB Agar	Metallic Sheen colonies	Rod
Aeromonas Agar	Agar Pale pink smooth, elevated Colony	Rod
BSA AGAR	Yellowish colour colonies	Cocci

Table: 2 Biochemical tests of different isolates.

Biochemical tests	Isolated bacterial colony*			
	W1	W2	W3	W5
Motility test	+	+	+	-
Indole test	+	_	+	-
Methyl red test	+	_	_	+
Voges- proskauer test	+	+	+	+
Citrate utilization test	_	_	+	+
Triple sugar iron agar	_	G	_	-
Urease hydrolysis test	+	_	_	+
Oxidase test	_	_	_	-
Catalase test	_	+	+	+
Carbohydrate Fermentation Test: a) Maltose test	+	_	+	+
b) Sucrose	+/G	_	+	+
d) Glucose	+	_	+	+
e) Adonitol	+	+	+	+
f) Arbanose	+	_	+	-
g) Lactose	_	+	+	+
i) Rhamnose	+	_	+	-
h) Sorbitol	+	_	+	-

*Based on the results of the colonies W1, W2, W3, W4

Table: 3 Isolated Bacteria

Media	Gram stain	Bacteria
EMB	Gram negative	*E. coli*
Aeromonas agar	Gram negative	*Aeromonas sp.*
TCBS	Gram negative	*Vibrio sp.*
BSA AGAR	Gram positive	*Staphylococcus aureus*

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Fig. 1. Gram Positive and Gram Negetive isolates from fish

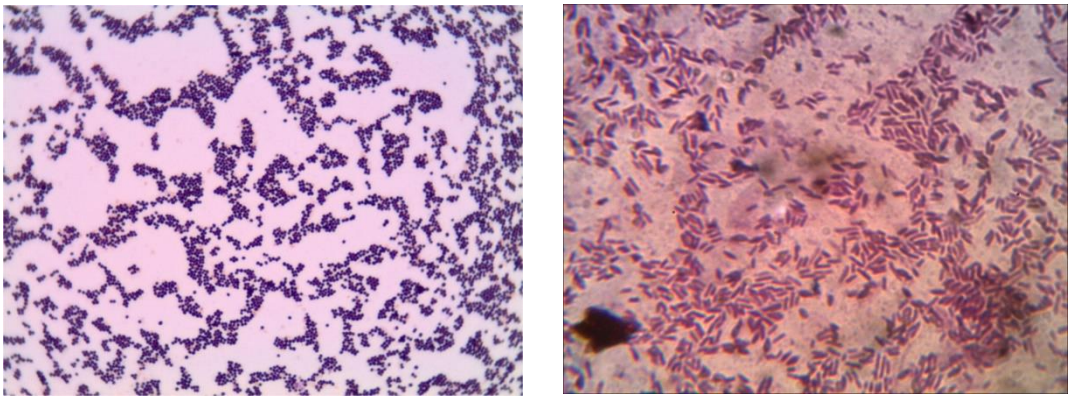

Fig. 2. Infected fish Clarius batrachus.

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